Genome-wide in vivo screen identifies novel host regulators of metastatic colonisation

Genome-wide *in vivo* screen identifies novel host regulators of metastatic colonisation

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Metastasis is the leading cause of death for cancer patients. This multi-stage process requires tumour cells to survive in the circulation, extravasate at distant sites, then proliferate, and involves contributions from both the tumour cell and tumour microenvironment ('host', which includes stromal cells and the immune system [1]). Studies suggest the early steps of the metastatic process are relatively efficient, with the post-extravasation regulation of tumour growth ('colonisation') being critical in determining metastatic outcome [2]. Here we show the results of screening 810 mutant mouse lines using an *in vivo* assay to identify microenvironmental regulators of metastatic colonisation. We identify 23 genes, that when disrupted in mouse, modify the ability of tumour cells to establish metastatic foci, with 19 of these genes not previously demonstrated to play a role in metastasis. The largest reduction in pulmonary metastasis was observed in sphingosine-1-phosphate (S1P) transporter Spinster Homolog 2 (*Spns2*)-deficient mice. We demonstrate a novel outcome of S1P-mediated regulation of lymphocyte trafficking, whereby deletion of *Spns2*, either globally or in a lymphatic endothelial-specific manner, creates a circulating lymphopenia and a higher percentage of effector T cells and NK cells present in the lung. This allows for potent tumour cell killing, and an overall decreased metastatic burden.

To identify microenvironmental genes that regulate metastatic colonisation we performed an 'experimental metastasis assay' involving

intravenous injection of B16-F10 mouse metastatic melanoma cells, used previously in the development of checkpoint inhibitors such as CTLA4 and PD-1 [3,4], and the assessment of pulmonary colonisation (**Fig. 1a**). The 810 mutant mouse lines we assayed were randomly selected and cover a diverse range of molecular functions (**Fig. 1b** and **SI Table 1**). Using a stringent 2-stage selection process, we identified 23 mutant lines showing significantly decreased or increased numbers of pulmonary melanoma foci, defined as a ratio of ≤ 0.6 or ≥ 1.6 and $P \leq 0.0175$ (Mann-Whitney test) for mutant mice versus wildtypes assayed concurrently (in the initial cohort assayed (**Fig. 1a**)), and $P < 0.01$ in an Integrative Data Analysis performed on 3 or more additional cohorts (**Fig. 1c**, **SI Table 2** and **Methods**). Since these strains were extensively phenotyped [5], we were able to determine that alterations of immune-related phenotypic traits featured prominently in these 23 mutant lines (**Fig. 1c**), highlighting the key role of the immune system in microenvironmental regulation of metastasis.

Of the 8 genes identified as suppressors of pulmonary metastases, two were members of the interferon regulatory family (IRF), important for immune function; loss of *Irf1* or *Irf7* increased pulmonary metastasis (as well as extra-pulmonary metastases in *Irf1*$^{tm1a/tm1a}$ mice), likely related to defects in their type-I IFN-dependent response [6,7]. In contrast *Irf5*-deficient mice, with their largely intact type-I IFN response [8], showed no altered pulmonary metastasis phenotype (**Extended Data Fig. 1)**. Similarly, the increased metastasis seen in p110 catalytic subunit of phosphoinositide 3-kinase (*Pik3cg*)-deficient mice is likely related to the critical function of

this gene in multiple aspects of T cell, NK cell and neutrophil function [9,10], and the increased metastasis seen in immunoglobulin heavy chain 6 (*Ighm*)-deficient mice is likely due to their multiple immune system abnormalities [11]. In contrast, very little is known about the other four genes we identified as microenvironmental suppressors of metastasis, namely *Abhd17a, Dph6*, *Slc9a3r2* and *Rnf10*, which represent novel factors for further studies. Of the 15 mutant mouse lines we identified as having decreased pulmonary melanoma colonies, four have been previously described as having roles in regulating metastasis; *Entpd1 (Cd39), Nbeal2, Cybb* and *Hsp90aa1*, contributing to regulatory T-cell control of NK cells [12], platelet α-granule function [13], generation of phagocyte-derived oxygen radicals [14] and the chaperoning of client proteins involved in tumour progression [15], respectively.

We focused on the sphingosine-1-phosphate (S1P) transporter *Spinster homolog 2* (*Spns2*), as *Spns2*$^{tm1a/tm1a}$ mice showed the greatest suppression in the number of pulmonary metastatic melanoma foci, with *Spns2*$^{tm1a/+}$ mice showing an intermediate phenotype (**Fig. 2a**). Further, *Spns2*$^{tm1a/tm1a}$ mice showed reduced numbers of foci in the lungs after tail vein administration of lung CMT-167, colorectal MC-38 or breast EO771.LMB cancer cells (**Fig. 2b**), and decreased spontaneous pulmonary metastasis (both in number and size of metastatic foci) after subcutaneous administration of HCmel12-mCherry melanoma cells (**Fig. 2c** and **Extended Data Fig. 2a**). In contrast, there was no difference in the growth rate of the primary tumour between wildtype and *Spns2*$^{tm1a/tm1a}$ mice, either for HCmel12-mCherry melanoma cells or B16-BL6 melanoma

cells, and no difference in the spontaneous incidence of cancer in aged wildtype and *Spns2*$^{tm1a/tm1a}$ mice (**Extended Data Fig. 2b-d).** Tail vein administration of transformed melanocyte WT31 cells (**Fig. 2d**) and intra-splenic administration of B16-F10 cells (**Fig. 2e**) showed a reduced number of foci in the livers of *Spns2*$^{tm1a/tm1a}$ mice, suggesting the resistance to metastatic colonisation is not pulmonary-restricted.

S1P is a bioactive lipid mediator that plays important roles in diverse cellular functions such as cell proliferation, differentiation, migration and tumourigenesis [16]. Previous studies have demonstrated SPNS2 functions as a cell-surface S1P transporter that allows intracellular S1P to be secreted into the blood and lymph [17-19]. In agreement with previous studies [17,19], S1P was decreased in serum and increased in lungs of *Spns2*$^{tm1a/tm1a}$ mice (**Extended Data Fig. 3**). Although extracellular S1P is a key regulator of endothelial barrier homeostasis [20], vascular permeability/extravasation of Evans Blue dye in *Spns2*$^{tm1a/tm1a}$ mice was the same as controls (**Fig. 2f**), as was the arrival of B16-F10 cells in the lung 90 minutes after tail vein administration (**Fig. 2g**). However, a significant increase in the number of pulmonary B16-F10 cells showing evidence of apoptosis was observed after 12 hours (**Fig. 2h**), suggesting that the lungs of *Spns2*$^{tm1a/tm1a}$ mice represent a hostile environment for tumour cell engraftment. RNA-seq analysis comparing viable B16-F10 cells isolated from lungs 24 hours after their administration identified nine differentially expressed (up-regulated) genes (**Source Data Table 1**); six of these genes (*Pla2g16, Epsti1, Traf1, Glipr2, Marcksl1* and *Ccl5*) are known to be involved in pro-metastatic phenotypes of tumour

cells, and H2-Q7-positive B16-F10 cells have been shown to be targeted by both NK and cytotoxic T-cells [21]. Thus, the transcriptional profile of B16-F10 cells from *Spns2*$^{tm1a/tm1a}$ lungs suggests they are upregulating genes to facilitate their survival in a hostile environment, whilst at the same time provoking activation of the immune system.

One of the most notable effects of S1P is the regulation of lymphocyte trafficking [22]. SPNS2 has been reported to function as an S1P transporter in endothelial cells but not in erythrocytes or platelets [17]. In agreement with others [17-19,23], *Spns2*$^{tm1a/tm1a}$ mice have a profound reduction in circulating T and B cells, with all other leukocyte (including NK cells) and blood cell lineages unaffected (**Extended Data Fig. 4a-c**). In the lung, the percentage of T cells was significantly reduced with a small reduction in the B cell percentage and increased NK cells (**Fig. 3a**), with similar phenotypes observed in the liver (**Extended Data Fig. 4d**). Consistent with *Spns2* expression in endothelial cells [17], bone marrow chimeras showed a lymphocyte and metastatic colonisation phenotype identical to the genotype of the host (**Fig. 3b** and **Extended Data Fig. 4e-f**), confirming that non-hematopoietic stroma regulates these observations. Expression of *Spns2* by endothelial cells is required for the maintenance of an S1P gradient in the lymph that is critical for regulating lymphocyte circulation [18]. In agreement with this, we showed that mice with lymphatic endothelial cell (LEC)-specific deletion of *Spns2* (*Spns2*$^{tm1c/tm1c}$; *Lyve1*$^{cre/+}$ mice) did not have altered serum or lung S1P levels (**Extended Data Fig. 5a-b**), yet displayed lymphopenia in the blood (**Fig. 3c),** lungs **(Fig. 3d)** and other tissues examined (**Extended Data**

Fig. 5c). Critically, this resulted in a decreased number of pulmonary metastasis in *Spns2*$^{tm1c/tm1c}$; *Lyve1*$^{cre/+}$ mice administered either B16-F10 or MC-38 cells (**Fig. 3e** and **Extended Data Fig. 5d**).

We next set out to establish the contribution of SPNS2 to the pulmonary immune microenvironment. S1P-S1PR1 signalling is essential for the recirculation of naive T-cells, however, memory T cells downregulate S1PR1 expression and rely on chemokine receptors for trafficking [24]. In contrast NK cell trafficking in response to S1P requires S1PR5 not S1PR1 [25]. In agreement with this differential requirement of S1P for trafficking, *Spns2*$^{tm1a/tm1a}$ mice showed a significantly higher percentage of anti-tumoral effector memory T cells ($CD44^{hi}CD62L^{lo}$) relative to immune suppressive regulatory T cells ($CD4^{+}CD25^{+}$) thus providing an enhanced effector:regulatory T cell ratio (**Fig. 3f and Extended Data Fig. 6a-b**), with the same observed in *Spns2*$^{tm1c/tm1c}$; *Lyve1*$^{cre/+}$ mice (**Fig. 3g and Extended Data Fig. 6c-d**). An increased proportion of activated T cells ($KLRG1^{+}$, $CD69^{+}$ and $CXCR3^{+}$) were also observed in the lungs of *Spns2*$^{tm1a/tm1a}$ and *Spns2*$^{tm1c/tm1c}$; *Lyve1*$^{cre/+}$ mice (**Fig. 3h-i**), with a similar phenotype seen in the liver **(Extended data Fig. 7**).

Consistent with this activated phenotype, we performed *ex vivo* restimulation assays, where T cells were isolated from the lungs of *Spns2*$^{tm1a/tm1a}$ and control mice 5 days after *in vivo* activation with B16-F10 cells. Using pharmacological stimulation both CD4 and CD8 T cells from *Spns2*$^{tm1a/tm1a}$ mice showed an enhanced degranulation response (cell surface expression of CD107a/LAMP1), and increased intracellular

interferon-γ (IFN-γ) relative to control mice (**Fig. 4a-b**). Interestingly, only CD8 T cells demonstrated enhanced degranulation when co-cultured with B16-F10 cells *ex vivo* suggestive of the presence of an improved antigen-specific response towards B16-F10 (**Fig. 4c**). This functionally resulted in enhanced B16-F10 target cell killing in an *ex vivo* cytotoxicity assay (**Fig. 4d**), and increased IFN-γ in lung lysates from B16-F10-stimulated *Spns2*$^{tm1a/tm1a}$ (**Fig. 4e**), and *Spns2*$^{tm1c/tm1c}$; *Lyve1*$^{cre/+}$ mice (**Fig. 4f**). Similarly, increased IFN-γ was also observed in lung lysates from MC-38-stimulated *Spns2*$^{tm1a/tm1a}$ mice (**Extended Data Fig. 8a**) indicating that this is not a B16-F10 restricted phenomenon. Although there was a significant increase in the relative proportion of NK cells in the lung, no difference in NK cell function could be observed *ex vivo* in *Spns2*$^{tm1a/tm1a}$ mice (**Fig. 4a-b**), in agreement with normal NK cell KLRG1, CD69 and CXCR3 expression in both *Spns2*$^{tm1a/tm1a}$ and *Spns2*$^{tm1c/tm1c}$; *Lyve1*$^{Cre/+}$ mice (**Extended Data Fig. 8b-c**).

To determine if the beneficial effects of *Spns2* in regulating metastatic colonisation could be mediated by CD8 T cells, we performed *in vivo* depletion experiments using anti-CD8 antibodies. However, paradoxically, depletion of CD8 T cells (or all T and B cells, such as in *Rag1* knockout mice) has previously been shown to decrease B16-F10 pulmonary metastasis (but not primary tumour growth); this phenomenon has been explained by the 'pro-tumoural' phenotype of CD8 T cells prior to tumour cell exposure versus the 'anti-tumoural' effect of antigen-specific CD8 T cells [26]. Indeed, we replicated this finding observing decreased pulmonary B16-F10 metastases in CD8-depleted wildtype

mice (**Fig. 4g**; and *Rag2* knockout mice, **Extended Data Fig. 9**), however, a genotype-specific effect was still observed in *Spns2*$^{tm1a/tm1a}$ mice, suggesting the involvement of additional cell types in the regulation of metastatic colonisation. Given that we observed compensatory NK cell activation (CD69^{+}) in the lungs of CD8-depleted *Spns2*$^{tm1a/tm1a}$ mice (**Fig. 4h**), we hypothesized NK cells could be responsible for the significantly reduced metastasis count compared to wildtypes. To explore this observation further, we performed NK cell depletion, resulting in increased B16-F10 metastases as reported previously [26], however *Spns2*$^{tm1a/tm1a}$ mice still showed a significantly reduced number of metastatic foci compared to wildtypes (**Fig. 4i**), in agreement with the enhanced CD8 response to B16-F10 cells observed *ex vivo* (**Fig. 4c**). To demonstrate the dual cellular identity responsible for protection in *Spns2*$^{tm1a/tm1a}$ we co-depleted NK and CD8 cells *in vivo* restoring the number of metastatic foci observed in *Spns2*$^{tm1a/tm1a}$ mice to those of wildtype (**Fig 4i**). Thus, we demonstrate that both CD8 T-cells and NK cells can contribute to the reduced pulmonary metastatic burden observed in *Spns2*$^{tm1a/tm1a}$ mice. An alteration of lymphatic endothelial cell function or lung sphingolipid levels in *Spns2*-deficient mice may also contribute to the reduced pulmonary metastatic burden we observe.

Finally we sought to manipulate the S1P system pharmacologically by inhibiting the S1P lyase that degrades S1P using 4'-deoxypyridoxine (DOP), a compound previously shown to increase lymphoid tissue S1P levels and induce a circulating lymphopenia [22]. DOP treatment phenocopied the immune and pulmonary metastasis phenotype of

Spns2$^{tm1a/tm1a}$ mice (**Extended Data Fig. 10)**, further validating the importance of the S1P axis in control of pulmonary metastatic burden. Importantly modulation of SPNS2 could be a more favourable approach than the S1P-blocking antibody Sphingomab[27,28] or the prodrug FTY720 [24] (which is phosphorylated *in vivo* to a functional antagonist of S1PR1) as these interventions increase regulatory T cell activity, suppress proliferation of effector T cells [29,30], and also increase vascular permeability [18]. Furthermore, as lymphatic endothelial cell-specific deletion of *Spns2* is sufficient to regulate lymphocyte circulation to allow a higher percentage of effector T cells and NK cells in the lung (and liver) and more tumour cell killing, targeting SPNS2 is potentially a more favourable option for regulating metastatic colonisation than existing S1P pathway modulators.

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AUTHOR CONTRIBUTIONS

L.v.d.W. devised and implemented the pulmonary metastasis screen performing all the primary screen, confirmation and characterisation studies. M.J.A. analysed the histopathological sections. A.D.C. and O.J.S. performed and analysed the intrasplenic B16-F10 assays. T.B. and T.T performed and analysed the spontaneous metastasis assay. H.W-J and N.G. managed mouse breeding and were responsible for issuing phenotyping cohorts. M.D.C.V-H., T.V., I.C.M. and K.W. performed the RNA-seq analysis. D.G. and E.R. genotyped the mice and performed gene expression analysis. S.C., A.G., E.T.

and E.L.C. performed additional phenotypic characterisation. The Sanger Mouse Genetics Project generated and phenotyped the mice as part of a primary phenotyping pipeline. S.S. oversaw the lipidomic analysis and provided input to the project and the manuscript. A.O.S. devised, performed and analysed the immunophenotyping assays. L.v.d.W., A.O.S. and D.J.A. led the project. L.v.d.W., A.O.S. and D.J.A. wrote the manuscript with contributions from all authors.

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Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to L.v.d.W (lvdw@sanger.ac.uk) or D.J.A. (da1@sanger.ac.uk).

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FIGURE LEGENDS

Figure 1 | Identification of microenvironmental regulators of metastatic colonisation of the lung. a, Experimental model (schematic) and results from stage 1 of the screen: experimental metastasis assay performed on 810 mutant mouse lines (detailed in the Extended Methods). Those lines with a metastatic ratio of ≤ 0.6 (red box) or ≥ 1.6 (green box) and Mann-Whitney test $P \leq 0.0175$ were taken forward to stage 2 (see below). **b**, Molecular function gene ontology annotation of the 810 mutant mouse lines screened as detailed in Materials and

Methods. **c**, Top level mammalian phenotype ontology terms for the 23 statistically significant genes following an Integrative Data Analysis of experimental metastasis assay results from three or more additional cohorts (green is increased metastasis and red is decreased). In the heatmap red boxes indicate a phenodeviant call, and blue no phenotype annotated (either no phenotype detected or not assayed) as detailed in Methods.

Figure 2 | Ability of *Spns2*-deficient mice to regulate metastatic colonisation. a, Experimental metastasis assay using B16-F10 cells in +/+ (blue), *tm1a/*+ (green) and *tm1a/tm1a* (red) male mice, with representative images. **b**, Experimental metastasis assay using CMT-167 (+/+, n=8; *tm1a/tm1a*, n=6 female mice), MC-38 (+/+, n=10; *tm1a/tm1a*, n=5 male mice) and EO771.LMB cells (+/+, n=12; *tm1a/tm1a*, n=5 female mice). **c**, Spontaneous metastasis assay using HCmel12-mCherry melanoma cells in male mice (n=10 per genotype), with representative fluorescent images (lines indicate the edge of the lungs). **d**, Experimental metastasis assay using WT31 transformed melanocytes in +/+ (n=18) and *tm1a/tm1a* (n=6) male mice. **e**, Intra-splenic administration of B16-F10 cells in +/+ (n=16) and *tm1a/tm1a* (n=15) female mice. **f**, Micrograms of extravasated Evans Blue dye in the lungs of +/+ and *tm1a/tm1a* male mice. **g**, Number of CFSE-labelled B16-F10 cells present in the lungs of female mice 90 min after administration. **h**, Levels of apoptosis in B16-F10-mCherry cells 12 hours after administration to male mice. Shown are representative data from 2 (CMT-167, **b**) or 3 independent experiments (**a**, **b** (MC-38 and EO771.LMB), **d**, **f-h**) or cumulative results of 2 independent experiments (**c, e**) with mean ± SEM (**b** - **e**) or symbols

representing individual mice with horizontal bar at the mean (**a**, **f**-**h**). *P* values are indicated from one-way ANOVA with Sidak's multiple comparisons adjusting for multiple testing (**a**), Mann-Whitney test (**b**-**d**), one-tailed unpaired t test (**e**) or two-tailed unpaired t test with Welch's correction (**f**-**h**).

Figure 3 | Characterisation of the lymphocyte composition and phenotype in *Spns2* mice. a, The percentage of lymphocyte subsets in the lungs of +/+ and *tm1a/tm1a* female mice. **b**, Number of metastases in B16-F10-dosed male bone marrow chimaeras (genotypes: +/+ (*WT*) and *tm1a/tm1a* (*KO*)). **c**, Numbers of lymphocytes in the blood of control and *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice. **d,** The percentage of lymphocyte subsets in the lungs of control and *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice (two-tailed unpaired t test adjusted by the Holm-Sidak method with alpha set to 5%). **e**, Experimental metastasis assay using B16-F10 cells in control and *tm1c/tm1c; Lyve1*$^{cre/+}$ female mice. **f-i**, T cell subset populations in the lungs of +/+ and *tm1a/tm1a* female mice or control and *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice. Shown are representative data from 2 (**b**) or 3 independent experiments (**a**, **c-i**) with symbols representing individual mice with horizontal bar at the mean (**a**-**i**). *P* values are indicated from two-tailed unpaired t test adjusted by the Holm-Sidak method with alpha set to 5% (**a, c-d, f-i**) or Mann-Whitney test (**b, e**).

Figure 4 | Lymphocyte activity within the lungs of *Spns2* mice. a-b, *Ex vivo* restimulation (PMA/ionomycin) of pulmonary leukocytes from B16-F10-stimulated +/+ and *tm1a/tm1a* female mice. **c**, Degranulation assay on

pulmonary leukocytes from B16-F10-stimulated +/+ and *tm1a/tm1a* female mice in response to *in vitro* restimulation with B16-F10. **d**, Cytotoxicity assay on pulmonary leukocytes from B16-F10-stimulated +/+ and *tm1a/tm1a* female mice (n=8 per genotype). **e-f**, Measurement of IFN-γ in lungs of B16-F10-stimulated +/+ and *tm1a/tm1a* female mice, and control and *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice. **g**, Experimental metastasis assay using B16-F10 cells in +/+ and *tm1a/tm1a* female mice treated with either isotype or anti-CD8 antibody. **h**, The proportion of activated (CD69^{+}) NK cells present in the lungs of +/+ and *tm1a/tm1a* female mice dosed with isotype or anti-CD8 antibody. **i**, Experimental metastasis assay using B16-F10 cells in +/+ and *tm1a/tm1a* male mice treated with either isotype, anti-NK1.1 or anti-NK1.1 and CD8 antibody. Shown are representative data from 3 independent experiments with symbols representing individual mice with horizontal bar at the mean (**a**-**c, e-i**) or mean ± SEM (**d**). *P* values are indicated from two-tailed unpaired t test adjusted by the Holm-Sidak method with alpha set to 5% (**a-b**), two-tailed unpaired t test with Welch's correction (**c**, **e**-**f**, **h**), two-way repeated measures ANOVA with Sidak's multiple comparisons test for each effector:target ratio (**d**) or Mann-Whitney test (**g, i**).

METHODS

Mice. The generation and genotyping of *Spns2*$^{tm1a(KOMP)Wtsi}$ (referred to as *tm1a/tm1a*)[23], *Spns2*$^{tm1b(KOMP)Wtsi}$, *Lyve1*$^{tm1.1(EGFP/cre)Cys/J}$ *(referred to as Lyve1*^{cre}) mice [31] and *Rag2*^{tm1Fwa} mice [32] have been described previously. *Spns2*$^{tm1c(KOMP)Wtsi}$ (referred to as *tm1c/tm1c*) mice were generated from crossing *Spns2*$^{tm1a(KOMP)Wtsi}$ mice with Flp-deleter mice [33] and crossed to *Lyve1*^{cre} mice to generate experimental mice (*tm1c/tm1c; Lyve1*$^{cre/+}$*)* with littermates used as controls (*tm1c/+; Lyve1*$^{+/+}$*and tm1c/tm1c; Lyve1*$^{+/+}$; referred to as "controls"). The care and use of all mice in this study were in accordance with the UK Animals in Science Regulation Unit's *Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes*, the Animals (Scientific Procedures) Act (ASPA) 1986 Amendment Regulations 2012, and all procedures were carried out under a Home Office Project licence, which was reviewed and approved by the Sanger Institute's Animal Welfare and Ethical Review Body. Housing and husbandry conditions were as described previously [34], with the exceptions that a cage density of 1-6 mice per cage was used and mice were maintained on Mouse Breeders Diet (Lab Diets, 5021-3) throughout the study. Unless specified otherwise, all mice were used at 6-12 weeks of age.

General Experimental Design. For the majority of experiments, random allocation to treatment group was achieved through the process of Mendelian inheritance, with age- and sex-matched mice being selected across different litters and matings (to minimise potential litter and/or cage effects). The two exceptions are the NK cell depletion study and bone marrow chimaera study;

in these studies Mendelian inheritance was used to randomise assignment of animals to a genotype group and then within this block alternate allocation was used to assign treatment. Unless specified otherwise, the researcher was not blinded to the identity of the genotype and/or treatment of a mouse during any procedures due to this being written on the cage card. Pilot experiments were performed to determine sample size with adequate statistical power for all studies except the high throughput screen where this this was not possible due to the scale of breeding that would be required. For each procedure, exclusion criteria used are listed where applicable in the materials and methods. If no exclusion criteria are detailed, all data were included. The manuscript was prepared to meet ARRIVE reporting guidelines [35].

Cell lines. The mouse melanoma B16-F10 cell line was purchased from ATCC (CRL-6475™) and the highly metastatic mouse melanoma B16-BL6 cell line was purchased from the University of Texas, MD Anderson Cancer Center and authenticated by whole genome and transcriptome sequencing. The mouse lung carcinoma CMT-167 cell line was purchased from Sigma-Aldrich (10032302) and the other cell lines were obtained from the laboratories that generated them. Specifically, the metastatic mouse colorectal MC-38 cell line [36] was a kind gift from Lubor Borsig (University of Zurich, Switzerland), the metastatic mouse mammary cancer EO771.LMB cell line [37] was a kind gift from Robin L. Anderson (Peter MacCallum Cancer Centre, Australia), the metastatic HCmel12-mCherry melanoma cell line [38] was a kind gift from Thomas Tuting (University of Bonn, Germany), and the transformed mouse melanocyte WT31 cell line (*Tyr::Nras*$^{Q61K/°}$*; INK4a*$^{-/-}$) [39] was a kind gift from Owen Sansom

(Beatson Institute for Cancer Research, Scotland). None of the cell lines used appear in the International Cell Line Authentication Committee database. All cells (apart from WT31 cells) were maintained in DMEM with 10% (v/v) foetal calf serum and 2 mM glutamine, 100 U/ mL penicillin/streptomycin (with the addition of 20 mM HEPES for EO771.LMB cells) at 37ºC, 5% CO_2. WT31 cells were maintained in RPMI with 10% (v/v) foetal calf serum and 2 mM glutamine, 100 U/mL penicillin/streptomycin at 37ºC, 5% CO_2. All cell lines were screened for the presence of mycoplasma and mouse pathogens (at Charles River Laboratories, USA) prior to culturing and never cultured for more than 5 passages. The B16-F10-mCherry cells, stably expressing mCherry, were generated by co-transfection of B16-F10 cells with 4.5 µg of PB-CAGG-LUC-2A-mCherry-PURO-PB plasmid (a kind gift from David Ryan, Wellcome Trust Sanger Institute) and 0.5 of PBase-expressing plasmid using Fugene HD (Promega) according to the manufacturer's recommendations. After selection in 5 µg/mL puromycin (Gibco BRL) for 10 days, when cell sorting was performed (MoFlo™ XDP, Beckman Coulter) to select for those cells expressing high levels of mCherry and was maintained in 5 µg/mL puromycin.

Experimental metastasis assay. B16-F10 ($4x10^5$), CMT-167 ($1x10^5$), MC-38 ($4x10^5$), EO771.LMB ($4x10^5$) or WT31 ($2.5x10^6$) cells resuspended in 0.1 mL PBS were injected into the tail vein of 6- to 12-week-old sex-matched syngeneic control and mutant mice. After 10 days (or 30 days if WT31 cells were used) the mice were sacrificed, their lungs removed (or livers removed if WT31 cells were used), and the number of metastatic foci counted macroscopically (for B16-F10 and WT31 cells) or microscopically from formalin-fixed H&E-stained

sections by a pathologist (for CMT-167, MC-38 and EO771.LMB cells; the pathologist was blinded to the genotypes of the samples). For intrasplenic injections of B16-F10 melanoma cells the mice were anaesthetised under isofluorane gas, with laparotomy performed to expose the spleen. B16-F10 cells ($1x10^4$) resuspended in 0.03 mL PBS were injected into the tail of the spleen, after which surgical incisions sutured and surgical clips applied. Animals monitored throughout recovery with dietary support and analgesia (Rimadyl 100 µg/mL ad libitum) provided. After 14 days, the mice were sacrificed, their livers removed, and the number of metastatic foci counted macroscopically.

Metastatic colonization screen. The experimental metastasis assay (detailed above) was performed by administering $4x10^5$ B16-F10 cells to age- and sex-matched wildtype and mutant mice. The mice were 6-12 weeks old (typically 6-8 weeks) at time of dosing and dosing cohorts typically consisted of 12-24 control mice with 3-5 different mutant alleles being screened (3-8 mutant mice per allele). To ensure consistency, preparation of the cells, administration into the tail vein and counting of pulmonary metastatic foci were performed by the same individual. To ensure a high level of accuracy, a two-stage process was implemented, with final calls only being made after data had been collected from multiple independent cohorts (the data from all mice were included in the analysis except for when the full 0.1 mL of cell suspension was not successfully administered due to difficulties at the time of injection). The first stage was a high throughput process to identify lines of potential interest for the second stage; in this stage, mutant lines with a ‘metastatic ratio’ (mean number of

metastatic foci in the mutant cohort divided by mean number of metastatic foci in the wildtype cohort) ≤ 0.6 or ≥ 1.6 and $P \leq 0.0175$ in the Mann-Whitney test (a subsequent analysis estimated a false discovery rate of 15%) progressed to the second stage. In the second stage, at least 3 additional cohort(s) of mice (of both sexes) were independently studied and the data combined into an Integrative data analysis (IDA) as detailed in the Statistics section.

Bone marrow chimaeras. Wildtype and *tm1a/tm1a* mice were given 2 x 4.2 Gy whole body irradiation followed by tail vein administration of $3x10^6$ bone marrow cells from either wildtype or *tm1a/tm1a* mice. Six weeks post-transplant a tail vein blood sample was taken from the mice for assessing the relative proportion of CD45.1 versus CD45.2 cells and number of T and B lymphocytes present in the peripheral blood and two days later an experimental metastasis assay performed.

***In vivo* depletion studies.** Mice were given as intra-peritoneal dose of antibodies (anti-CD8 [clone YTS169.4)], rat IgG2b isotype control [clone LTF-2]), anti-NK1.1 [clone PK136], mouse IgG2a [clone C1.18.4]), 200 µg in 0.1 mL PBS on day -3, 0 and +5, with B16-F10 cells tail vein administered on day 0 (CD8-depletion mice were dosed with $4x10^5$ B16-F10 cells; NK and NK/CD8-depletion mice were dosed with $2x10^5$ B16-F10 cells). Tail vein blood samples were collected from all mice on day +1 to confirm the depletion was effective. All antibodies were “InVivoMAb” from BioXCell.

S1P lyase inhibitor studies. For S1P lyase inhibitor studies, the mice were either given glucose (10 g/L) or glucose plus 4' deoxypyridoxine (DOP, 30 mg/L; Sigma) in their drinking water 1 week prior to any experimentation (with mice remaining on it for the duration of the experiment) [22].

Primary tumour growth studies. For examination of orthotopic tumour growth, wildtype and *tm1a/tm1a* male and female mice at 6-8 weeks of age were subcutaneously administered $2.5x10^3$ B16-BL6 melanoma cells in the flank. The developing tumours were measured every second day and if they had reached (or were very close to) $2cm^2$ on the day of measurement the mice were immediately culled (no tumour was ever more than $2.4cm^2$), as approved by the Home Office Inspector under the authority of ASPA.

Spontaneous metastasis assay. Wildtype and *tm1a/tm1a* mice were subcutaneously dosed with $2x10^5$ HCmel12-mCherry melanoma cells and the resulting tumour growth was monitored by inspection and palpation. The size of the tumour was measured weekly using vernier calipers and recorded as mean diameter. Mice were killed when progressively growing melanomas exceeded 20 mm in size and tissues collected for further analyses (in accordance with institutional and national guidelines for the care and use of laboratory animals with approval by the local government authorities (LANUV, NRW, Germany)). The number of macroscopically visible metastases present on the lung surface were counted by two independent investigators in a blinded fashion.

Preparation of tissue cell suspensions. Mice were perfused with 20 mL phosphate buffered saline (PBS) by cardiac puncture and the tissues were disrupted in C tubes using program m_lung_01 with an gentleMACS (Miltenyi Biotec) in Hanks Balanced Salt solution (HBSS) containing calcium and magnesium. Liberase DL (Collagenase with low dispase content, Roche, Burgess Hill, UK) was added to a final concentration of 0.1 U/mL and incubated for 30 min at 37^{o}C. The tubes were then processed using program m_lung_02 and DNase (0.1 mg/mL) was added for a further 30 min at 37^{o}C. The resulting cell suspension was centrifuged at 400 x *g* for 5 min, resuspended in 2 mL FACS buffer (D-PBS without calcium and magnesium containing 2 mM EDTA, 0.5% fetal calf serum and 0.09% sodium azide), passed through a 30 µm cell strainer and analysed on the flow cytometer. For determining the number or viability of melanoma cells present in the lungs of mice, the mice were dosed with either 1x10^{6} B16-F10 cells labelled with 10 µM CFSE (Molecular Probes, Invitrogen) at 90 min prior to perfusion or 1x10^{6} B16-F10-mCherry cells at 12 hrs prior to perfusion. In each case, the lung cell suspension was analysed on the flow cytometer. For lung/liver leukocyte analysis, the leukocytes were enriched from other cell types in the cell suspension on a Percoll™ discontinuous gradient (67.5%/44%) and washed three times with FACS buffer. Single cell suspensions from spleen and lymph nodes (pooled inguinal) were prepared using frosted end of microscope slides in FACS buffer. Red blood cells were lysed from spleen samples by the addition of 2 mL 1x PharmLyse (BD Biosciences) for 90 seconds at room temperature then stopped by the addition of 10 mL FACS buffer. Both spleen and lymph node samples were passed through a 30 µm cell strainer prior to staining. ‘Naïve’ mice were those

that had not been administered B16-F10 cells and 'stimulated' mice were those that had been tail vein administered B16-F10 cells 3 or 5 days prior to analysis as indicated in the figure legend.

FACS immunostaining. Samples were blocked with 1 µg of Mouse BD FC Block™ (anti CD16/32, clone 2.4G2, BD Biosciences) for 10 min prior to addition of multicolour antibody cocktails using titrated amounts to give saturating binding (see **SI Table 3** for more details). After washing, cells were stained with a viability dye (Live/Dead Blue, Invitrogen, 1 in 1000 dilution in PBS) for 10 min at room temperature before being washed prior to acquisition. For apoptosis determination lung preparations were prepared as above and were stained with Caspglow reagent (eBioscience UK) according to the manufacturers instructions for 1 hr at 37^{o}C. Cells were washed with Annexin binding buffer and stained with Annexin V-APC (both BD Biosciences) according to the manufacturers instructions for 15 min at room temperature. Cells were washed with Annexin binding buffer and resuspended Annexin V binding buffer containing 1 µg/mL DAPI (Life Technologies) prior to acquisition. For the determination of absolute cell counts of leukocyte populations, whole blood was counted with a haematology analyser (Scil Vetabc) and the white blood cell count was used to derive the cells/µL blood with the immune cell populations as % of leukocytes.

Lung leukocyte cytotoxicity. Leukocytes were prepared from perfused lungs 5 days post B16-F10 injection as described in 'experimental metastasis assay' above. B16-F10 target cells were labelled with 1 µM CFSE (Molecular Probes,

Invitrogen). Target cells and lung leukocytes were added to 96 well round bottom plates at effector to target ratios indicated for four hours at 37^{o}C in complete DMEM medium (prepared as described in cell lines section). The cells were washed twice with ice-cold PBS then resuspended in 100 µL Live/Dead far red (Invitrogen, 1 in 1000 dilution in PBS) for 10 min at room temperature. Cells were washed twice and resuspended in BD Cell Fix for 10 min at room temperature and washed twice with FACS buffer, prior to acquisition where 2,000 target cells were collected. Cytotoxicity was calculated according to the following equation [% of dead target cells with effector cells] – [% of dead target cells with no effector cells added].

Leukocyte degranulation and IFN-γ production. Leukocytes were prepared from perfused lungs 5 days post B16-F10 injection as described in 'experimental metastasis assay' above. Cells were stimulated with target cells (B16-F10 at effector to target ratio of 2.5:1) or phorbol myristate acetate (PMA) and ionomycin (100 ng/mL and 150 ng/mL, respectively both Sigma-Aldrich). Cells and stimulus were added to 96 well round bottom plates in the presence of anti-CD107a antibody and BD GolgiStop™ (monensin, final concentration 2 µM) in complete DMEM medium for four hours at 37^{o}C. The plates were washed twice with ice-cold FACS buffer before blocking then staining with anti-TCRβ, CD45, NK1.1 and CD8α antibodies. Cells were then stained with a fixable viability indicator (Live/Dead Blue, Invitrogen) prior to intracellular staining for IFN-γ according to standard methods and analysed by flow cytometry where a minimum of 50,000 CD45+ alive leukocyte events were collected. CD107a and IFN-γ gates were set on unstimulated leukocyte

samples and specific degranulation or intracellular IFN-γ staining was calculated by subtracting the leukocyte alone unstimulated values from the treated values.

Flow cytometry. All samples were analysed on a LSR II or LSRFortessa™ (both BD Biosciences) that were standardised using BD Cytometer Setup and Tracking beads and software. Compensation was determined using Ultracomp eBeads (eBioscience) for all antibodies and ArC amide binding beads (Invitrogen) for live/dead stains. Data acquisition was controlled with BD FACSDiva v6.3 or v8.0.1 software. For the analysis of B16-F10 apoptosis a threshold was applied to the mCherry channel (561nm laser 610/30 BP) to exclude 90% of the lung cells. For the analysis of cytotoxicity a threshold was applied to the CFSE channel (488nm laser 530/30 BP) to exclude 90% of the lung leukocytes. In both cases these were established using B16-F10 mCherry or CFSE labelled B16-F10 cells. In all other cases a FSC-A threshold was used to exclude debris. All samples were analysed using FlowJo 10.7 and were analysed genotype and/or treatment blind. For all phenotyping data doublets were excluded using FSC-A versus FSC-H gates, sample acquisition issues (such as clumps and unstable event rate) were excluded using a time gate against a fluorescent parameter that was off the laser with the longest time delay, dead cells were excluded from all tissue analysis using a viability indicator and debris excluded with FSC-A versus SSC-A gates. A leukocyte gate was set with CD45 and SSC-A and all cell subsets are reported as the percentage of this parent gate. T cells were defined as TCRγδ^{-} CD3^{+} NK1.1^{-} or TCRβ^{+} NK1.1^{-} with CD4^{+} and CD8^{+} gates defined on this parent population, NK

cells defined as NK1.1^{+} CD3^{-} or TCR^{-} and B cells defined as CD19^{+}. T and NK cell phenotypes were determined using fluorescent minus one controls to establish gating. Data from a sample was excluded if there were insufficient events in the parent gate to allow analysis, for example if there were less than 50,000 CD45^{+} alive leukocytes in lung phenotyping data this was excluded from the dataset.

Lung IFN-γ determination. Five days post-B16-F10 injection as described in 'experimental metastasis assay' above, lungs were saline perfused and homogenised in Tris buffered saline with 0.5% Triton X100 using M tubes and a gentleMACS (Miltenyi Biotec) with program protein_01. Samples were cleared by centrifugation for 10 min 20,000xg at 4^{o}C. IFN-γ levels in the lung lysates were determined using a Ready Set Go ELISA kit (eBioscience, Hatfield, UK) according to the manufacturer's instructions.

Transcriptome sequencing. Wildtype and *Spns2* homozygous mice tail vein dosed with 1x10^{6} B16-F10-mCherry cells were sacrificed after 24 hrs and lung cell suspensions prepared as described above. Using a cell sorter (MoFlo™ XDP), B16-F10-mCherry cells were identified after displaying in a bivariate plot of SSC-log versus mCherry by gating on high forward scatter versus side scatter to exclude some debris and dead cells and positively sorted. RNA was extracted from the sorted cells using the RNeasy Mini kit (Qiagen), according to the manufacturer's instructions and used to generate cDNA using the Smart-seq2 protocol [40,41]. Multiplexed sequencing libraries were generated from amplified cDNA using Nextera XT (Illumina). The multiplexed mRNAseq

libraries were pooled and sequenced across multiple lanes on the Illumina Hiseq 2000 (v3). Paired-end 100 bp reads were aligned with STAR version 2.3.0 [42] allowing a minimum and maximum intron size of 50-500000 bp, respectively. STAR genome index files were generated using a GTF file corresponding to gene models from ENSEMBL version 74 and reference genome version GRCm38. Read counting was performed with htseq-count from the HTSeq package (version 0.5.4p5) [43]. The htseq-count software was run with the options 'intersection-nonempty' mode, non-stranded, minimum quality 10, and 'exon' was used as the feature type, with 'gene_id' as the GTF feature ID. The Bioconductor (version 3.1) [44] package DESeq2 (version 1.8.1) [45] was used for differential expression analysis. We used the local fit parameter for dispersion fitting and obtained the significance with the DESeq2 negative binomial Wald test function. Genes with adjusted $P < 0.05$ after Benjamini-Hochberg correction and a $\log_2$ foldchange < -0.59 or > 0.59 were considered significantly differentially expressed.

Analysis of sphingolipids by LC-ESI-MS/MS. Serum and saline perfused lung tissues were collected from the mice. After the addition of internal standards (0.5 nmol each; Avanti Polar Lipids, Alabaster, AL, USA) the lipids were extracted and sphingolipids were quantified by LC-ESI-MS/MS (4000 QTRAP, AB Sciex, Framingham, MA, USA) as described previously [46].

Statistics. Statistical tests were selected to be appropriate for the data properties (e.g. normality or homogeneity of variance) and experimental design such the assumptions of the test would be met. Where multiple testing occurred

within a study, it was managed by controlling the family wise error rate as detailed in the associated figure legend. Integrative Data Analysis (also called Mega-Analysis) [47] was completed using R (package nlme v3.1) treating each experiment as a fixed effect. An iterative top down modelling strategy was implemented starting with the most comprehensive model (either Eq. [1], or [2]) appropriate for the collection strategy implemented and ensuring the model only included term where the terms could be independently assessed.

$$Y=\beta_0 + \beta_1 Sex + \beta_2 Experiment + \beta_3 Genotype + \beta_4 Sex*Genotype \quad [Eq.1]$$

$$Y=\beta_0 + \beta_2 Experiment + \beta_3 Genotype \quad [Eq. 2]$$

The optimisation process first selected a covariance structure for the residual, then the model was reduced by removing non-significant fixed effects, and finally the genotype effect was tested and model diagnostics visualised. For the hypothesis test of primary interest, the impact of genotype, the per-comparison error rate threshold *P* values were adjusted to account for the multiple comparisons to control the family wise error rate to 5% using the Hochberg method [48].

Bioinformatic analysis of molecular functions and phenotype. Using the Mouse Genome Informatics (MGI) portal (http://www.informatics.jax.org) all 810 mutant lines screened were separated into unique symbols (to separate out microRNA clusters) and annotated with molecular function using the Gene Ontology (GO) batch query selecting the GO_Slim annotations. Phenotypic information was pulled from MGI as a batch query (MGI 6.06 release date 5th October 2016) and supplemented with annotations from the International Mouse Phenotyping Consortium (IMPC – release 4.3 26th April 2016) portal

(http://www.mousephenotype.org). The reported mammalian phenotype (mp) terms returned were collapsed to the top-level term for the generation of the heatmap. We are not able to discriminate between no phenotype detected and no phenotypic data present, thus both outcomes are represented with a blue cell with the presence of phenotypes indicated by the red cell.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data for Extended Data Figure 2c is available on the online version of this manuscript. The data for the results of the experimental metastasis assay from stage 1 of the screen and the integrative data analysis (IDA) are available on the online version of this manuscript. All RNAseq data is available to access [ENA Study Accession Number: ERP005660; ArrayExpress Accession Number: E-ERAD-287] with the results of the analysis shown in Source Data Table 1 (available on the online version of this manuscript).

EXTENDED DATA FIGURE LEGENDS

Extended Data Figure 1 | Phenotyping mice deficient for members of the interferon regulatory factor (*Irf*) family. a, Experimental metastasis assay using B16-F10 cells in *Irf1*$^{tm1a/tm1a}$, *Irf5*$^{tm1e/tm1e}$, *Irf7*$^{tm1a/tm1a}$ and concurrent control female mice. Shown are representative data from 2 (*Irf5*), 4 (*Irf1*) or 6 (*Irf7*) independent experiments. Symbols represent individual mice with horizontal bar at the mean. *P* values are from a Mann-Whitney test. **b-e,** Representative photographs showing B16-F10 metastatic colonies on the **(b)**

lungs of +/+ and *Irf1*$^{tm1a/tm1a}$ mice and **(c-e)** the presence of extra-pulmonary metastases in *Irf1*$^{tm1a/tm1a}$ mice (tissues from 3 mice shown).

Extended Data Figure 2 | Spontaneous pulmonary metastases and primary tumour growth in *Spns2* mice. **a**, Size measurements of spontaneous pulmonary HCmel12-mCherry melanoma cell metastases of male mice (n=10 per genotype, horizontal bars represent mean (of 50 individual metastases counted per genotype), one-way ANOVA with blocking factor of experiment, cumulative results of 2 independent experiments shown). **b**, Survival curve of +/+ and *tm1a/tm1a* male mice (n=10 per genotype) in a spontaneous metastasis assay using HCmel12-mCherry cells (Log-rank test (Mantel-Cox), cumulative results of 2 independent experiments shown). **c**, Growth of subcutaneously administered B16-BL6 cells in +/+ (4 male, 5 female) and *tm1a/tm1a* (5 male, 1 female) mice. Symbols represent mean ± SEM with a two-tailed unpaired t test with Welch's correction used to compare the areas under the curve. **d**, Incidence of cancer in aged (>40 weeks) +/+ (n=15; 4 males, 11 females) and *tm1a/tm1a* (n=18; 5 males, 13 females) mice. Statistical analysis was performed using a Fisher's Exact test.

Extended Data Figure 3 | Analysis of sphingoid base levels in *Spns2* mice. Sphingoid base levels in the **a**, serum (+/+, n=5; *tm1a/tm1a*, n=4) and **b**, lungs (+/+, n=6; *tm1a/tm1a*, n=5) of male mice, data is mean ± SEM, multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%. Sph, sphingosine; DHSph, dihydrosphingosine; S1P, sphingosine-1-phosphate; DHS1P, dihydrosphingosine-1-phosphate.

Extended Data Figure 4 | Phenotypic characterisation of the haematopoietic system of *Spns2* mice. a-c, The numbers of erythrocytes and platelets, monocytes, granulocytes and lymphocytes subsets present in the blood of naïve +/+ and *tm1a/tm1a* female mice (multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%, data shown are representative of three independent experiments). **d,** Analysis of lymphocyte subsets in the liver of naïve +/+ and *tm1a/tm1a* female mice (multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%, data shown are representative of three independent experiments). **e-f**, T and B lymphocyte numbers in the blood of male naïve (unstimulated) bone marrow chimaeras (unpaired two-tailed t test with Welch's correction, data shown are representative of two independent experiments). Symbols represent individual mice and horizontal bars represent mean.

Extended Data Figure 5 | Characterisation of the phenotype of *tm1c/tm1c; Lyve1* mice. **a-b**, Sphingoid base levels in the serum (**a**) or lung (**b**) of control and *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice (data is mean ± SEM, multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%). Sph, sphingosine; DH-Sph, dihydrosphingosine; S1P, sphingosine-1-phosphate; DH-S1P, dihydrosphingosine-1-phosphate**. c**, Lymphocyte subsets in the spleen, lymph node, lung and liver of +/+ and *tm1a/tm1a* male mice (symbols represent individual mice, horizontal bars represent mean, multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with

alpha set to 5%, data shown are representative of three independent experiments). **d**, Experimental metastasis assay using MC-38 cells in control (n=9) and *tm1c/tm1c; Lyve1*$^{cre/+}$ (n=5) in female mice. Data shown are mean ± SEM, Mann-Whitney test, representative of 3 independent experiments.

Extended Data Figure 6 | T cell subsets in the lungs of *Spns2* mice. The proportion of T cell subsets present in the lungs of naïve +/+ and *tm1a/tm1a* female mice (**a-b**) and control and *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice (**c-d**). Symbols represent individual mice, statistical analysis was performed using multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%. Data shown are representative of three independent experiments.

Extended Data Figure 7 | T cell subsets in the liver of *Spns2* mice. The proportion of T cell subsets present in the liver of naïve +/+ versus *tm1a/tm1a* female mice and control versus *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice. Data are shown as percentage of parent $CD4^+$ and $CD8^+$ T cells (**a, c**, **e**, **f**) or percentage of $CD45^+$ alive liver cells present (**b**, **d**). Symbols represent individual mice, statistical analysis was performed using multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5% with * indicating *P* value that is not considered significant after correcting for multiple testing. Data shown are representative of three independent experiments.

Extended Data Figure 8 | Phenotyping of *Spns2* lungs. a, Measurement of IFN-γ in lungs of MC-38-stimulated +/+ and *tm1a/tm1a* male mice (unpaired

two-tailed t test with Welch's correction). **b-c**, The proportion of NK cell subsets present in the lungs of naïve +/+ versus *tm1a/tm1a* female mice (**b**) and control versus *tm1c/tm1c; Lyve1*$^{cre/+}$ male mice (**c**) (multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%). Symbols represent individual mice, horizontal bars represent mean and data shown are representative of three independent experiments.

Extended Data Figure 9 | Studies in T- and B-cell deficient mice. a, Measurement of lymphocyte subsets in the blood of +/+ and *Rag2*$^{-/-}$ mice (multiple two-tailed unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%). **b**, Experimental metastasis assay using B16-F10 cells in +/+ and *Rag2*$^{-/-}$ female mice (Mann-Whitney test). Symbols represent individual mice, horizontal bars represent mean and data shown are representative of three independent experiments.

Extended Data Figure 10 | Characterisation of the leukocyte composition and phenotype in 4' deoxypyridoxine (DOP)-treated mice. a-d, The number of leukocytes and T cell subsets present in the lungs of B16-F10 dosed glucose- or DOP-treated wildtype male mice presented as percentage of viable CD45^{+} lung leukocytes or percentage of parent CD4^{+} or CD8^{+} T cells (multiple unpaired t tests with *P* value adjusted by the Holm-Sidak method with alpha set to 5%). **e,** Experimental metastasis assay in B16-F10 dosed glucose- or DOP-treated wildtype female mice (Mann-Whitney test). Symbols represent individual mice, horizontal bars represent mean and data shown are representative of 2 independent experiments.

SUPPLEMENTARY INFORMATION TABLES

SI Table 1 | Results of the experimental metastasis assay from stage 1 of the screen. A mutant (Mut) mouse line progressed to stage 2 if the metastatic ratio was ≤ 0.6 or ≥ 1.6 and $P \leq 0.0175$ (Mann-Whitney test), and additional cohorts were tested. Where multiple cohorts were tested: (i) data is shown for the results from the first cohort, (ii) those with a significant results by Integrative Data Analysis ($P < 0.01$) are in red/green font (depending if decreased/increased metastasis), and (iii) those without significant results by Integrative Data Analysis ($P > 0.01$) are in blue font. *P*- and *U*-value listed in the table is from a Mann-Whitney test. F, female; M, male; Mut, mutant; WT, wildtype.

SI Table 2. Results of the Integrative Data Analysis (IDA). IDA was performed on experimental metastasis assay data from mutant mouse lines tested in ≥2 independent cohorts. Shown in the top box are mutant lines that were significant from stage 1 of the screen (39/810, 5%), and were found to be significant by IDA ($P < 0.01$; 23/39, 59%); termed “hit”. Green indicates mice with an increased metastatic burden and red indicates mice with a decreased metastatic burden (the magnitude of the burden is indicated by the "genotype effect"). The genes are listed in order of "genotype effect". Shown in the middle box (in blue) are mutant lines that were significant from stage 1 of the screen, and were not found to be significant by IDA ($P > 0.01$; 16/39, 41%); termed

“false positive”. Shown in the bottom box (in black) are mutant lines which were not significant from stage 1 of the screen (102/810, 12%) for which additional cohorts were tested to ensure the selection criteria from stage 1 was not too stringent; termed “repeated negative”. F, female; M, male; SE, standard error.

SI Table 3. Details of the antibodies used in this study for flow cytometry.

SOURCE DATA

Source Data Table 1. Results of analysis of RNAseq data. Differentially expressed genes in B16-F10-mCherry cells isolated from the lungs of *tm1a/tm1a* mice relative to those from +/+ mice 24 hours after administration (n=5 per genotype).